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ULTRASTRUCTURE OF HUMAN CORNEAL NERVES

Linda J. Müller[1], Liesbeth Pels[1], Anneke de Wolf[1], Gijs F.J.M. Vrensen[1] and Mike Kliffen[2].
[1] Department of Morphology, The Netherlands Ophthalmic Research Institute, Amsterdam (NL).
[2] Department of Ophthalmology, Erasmus University, Rotterdam (NL)

Purpose: The human cornea is a densely innervated structure. However, data on nerve fiber (NF) distribution in the human cornea are scarce possibly due to fast post mortem degeneration. Therefore, most descriptions on corneal innervation are based on studies in rats and rabbits. The present study is focussed on the ultrastructure of NF's in the central and peripheral human cornea.
Methods: Tissue samples of five corneas obtained from melanoma eyes were processed for light and electron microscopy (EM). Both frontal and cross-sections of stroma and epithelium were studied. Ultrathin stromal and ultrathin serial sections between Bowman's Membrane (BM) and the basal epithelium were cut. Stromal and subepithelial axon diameters were measured on EM micrographs.
Results: Unmyelinated NF's containing both clear and dense cored vesicles run parallel to the collagen fibers in the upper third of the central stroma. They consist of up to 30 fibers (diam. 0.5-2.5 μm) which are ensheathed by thin rims of Schwann cell protrusions and amorphic matrix. Some of these NF's, containing glycogen particles, invaginate the cytoplasm of the stromal keratocytes. After passing through BM, central stromal NF's (diam. 0.05-0.5 μm), run parallel between BM and epithelium. At the location of the varicosities, fibers measure up to 2 μm due to the presence of many mitochondria. These fibers turn upwards and protrude mainly into the basal cells and sometimes into the wing cells. Reconstruction of NF's in semithin sections revealed that subepithelial fibers, having one or more bifurcations, run in a 12h-6h direction in the centre and in a 3h-9h direction in the periphery. In contrast to the central cornea, myelinated fibers were only found in the peripheral limbal stroma.
Conclusion: NF's invaginating epithelial cells and keratocytes suggest that both epithelium and stromal keratocytes are directly innervated. The numerous mitochondria present in the varicosities point at fibers from sensory nerves. Therefore, the conclusion seems to be justified that the presence of clear and dense cored vesicles in NF's of stroma and subepithelium point at either an adrenergic or an cholinergic input from sensory origin.

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MACULAR CORNEAL DYSTROPHY IN ICELAND:
A IMMUNOHISTOCHEMICAL AND GENEALOGICAL STUDY OF 28 CASES.

JONASSON, F.[1], OSHIMA, E.[2], KLINTWORTH, G.K.[2], THONAR, E.J-A.[3], SMITH, C.F.[2], JOHANNSSON, J.H.[4].
1. University Department of Ophthalmology, Landakot Hospital, 101 Reykjavík, Iceland.
2. Departments of Ophthalmology and Pathology, Duke University Medical Center, Durham, North Carolina.
3. Departments of Biochemistry and Internal Medicine, Rush Medical College at Rush Presbyterian-St.Luke's Medical Center, Chicago, Illinois.
4. Department of Pathology, University of Iceland.

Purpose of the study is to determine what types of macular corneal dystrophy (MCD) are predominant in Iceland and if immunohistochemistry can be used for carrier detection.

METHODS: Using a ELISA which uses an anti-keratan sulfate (KS) monoclonal antibody, we measured serum levels of antigenic KS in 27 patients with MCD and 53 clinically unaffected family members. We stained sections from 37 corneal buttons from 23 MCD patients by avidin-biotin complex method using the same anti-KS monoclonal antibody.

RESULTS: 22 of the 28 patients with MCD had no detectable antigenic KS in serum; 5 had normal levels and one patient died before serum could be assayed. Corneas from patients with no detectable antigenic KS in serum lacked immunohistochemical reactivity to the anti KS antibody. All unaffected family members carrying the recessive gene had normal serum antigenic KS levels.

CONCLUSIONS: MCD types I (78.6%) and II (21.4%) both occur in Iceland. Members of affected sibships only had one of these types. Nine cases of MCD type I and 4 persons with MCD type II belong to a large pedigree with common ancestors in the 16th century. This inbread pedigree is consistent with two independant mutant genes though it may also present a different manifestation of the same genetic defect. Determining serum levels of antigenic KS was not helpful in carrier detection.

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EVIDENCE OF CORNEAL LATENCY FOLLOWING HERPES SIMPLEX VIRUS DNA DETECTION BY IN SITU HYBRIDIZATION

SALGADO-BORGES J.[1,2], SILVA-ARAÚJO A.[1,2], TAVARES M.A.[2,3] and ALVES V.[4]
1. Department of Ophthalmology, Medical School of Porto / H. S. João; 2. Institute for Molecular and Cellular Biology (IBMC), University of Porto; 3. Institute of Anatomy, Medical School of Porto; 4. Department of Microbiology, H.S. João, Porto (Portugal).

Purpose : The aim of this study was to determine whether Herpes Simplex Virus (HSV) DNA was present in quiet corneas of patients submitted to penetrating keratoplasty after an asymptomatic period of at least 8 months.

Methods : The ENZO PathoGene^R Probe Assay for HSV identification (a simple and straightforward procedure to detect and identify HSV in cells or tissues under conditions that preserve cellular morphology) was performed in the corneal buttons of 60 patients submitted to penetrating keratoplasty (PK) during the last 3 years: 20 patients undergoing PK for prior herpes simplex keratitis (group A), 20 patients with non-herpetic infectious leucomas (group B) and 20 patients with keratoconus (group C).

Results : No positivity was detected in any of the patients with keratoconus. In group B one cornea disclosed a positive reaction. In 7 out of the 20 corneas from patients of group A the presence of herpes simplex viral DNA was identified by the in situ hybridization procedure.

Conclusions : Our study suggests that HSV may be mantained in a latent state in corneas of patients with herpes simplex keratitis and even rarely in some patients with corneal disease non-related to the herpes simplex virus.

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Corneal Gelatinase A : Characterisation and activation.

Smith VA and Easty DL.
Department of Ophthalmology, Bristol Eye Hospital, Bristol BS1 2LX.

Chronic, progressive ocular diseases that involve disruption of the corneal matrix include keratoconus and peripheral ulcerative keratitis. A common feature of these diseases is the over-production of a matrix-metalloprotease (MMP-2, Gelatinase A) that exhibits specificity for Type IV collagen, the major component of basement membranes. Under normal circumstances, the activity of this enzyme is held under stringent control and a single activity band, of apparent MW 65,000 is visualised on gelatin-polyacrylamide gels. In the diseased tissues it is presumed that a regulatory mechanism has failed and an additional activity, of apparent MW 61,000 is produced. We are investigating control mechanisms that are specific to corneal Gelatinase A production and activation. Results obtained to date show that the resolved activities differ in conformation not in mass, that neither conformer is active as secreted, that part of the activation mechanism may involve autocatalysis and finally, that the ability to selectively hydrolyse Type IV collagen is associated with a protein of MW 43,000.